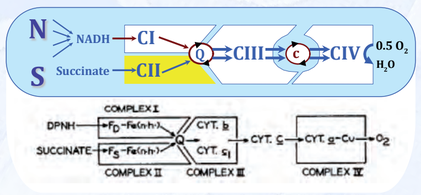
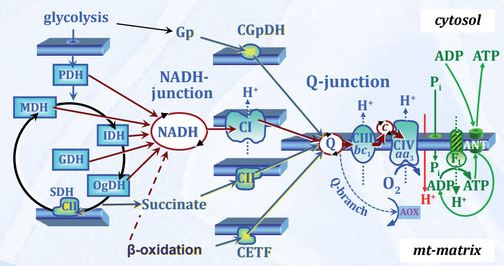
MitoPathways: OXPHOS analysis

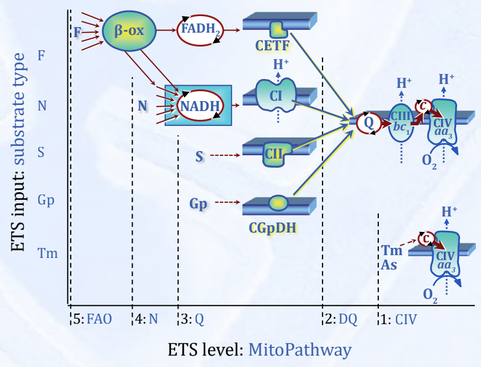
OROBOROS INSTRUMENTS

Convergent architecture of the electron transfer system: NADH- and Q-junction



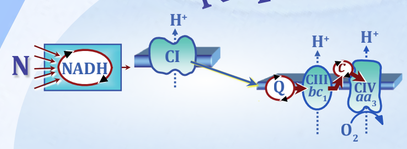
Hatefi et al (1962) JBC - Gnaiger E (2009) IJBCB



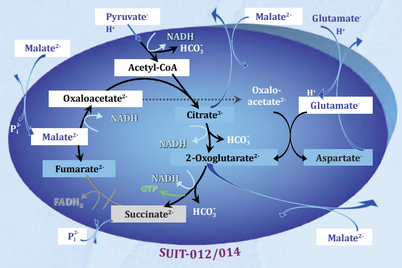


ETS level: MitoPathway

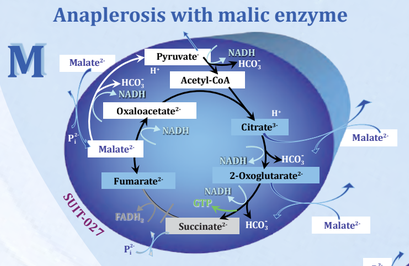
N-pathway: CI-linked



PGM



Anaplerosis with malic enzyme



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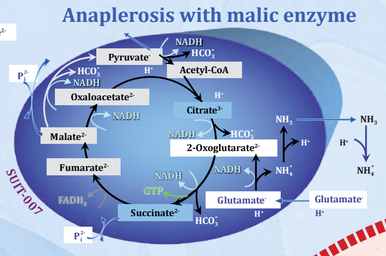
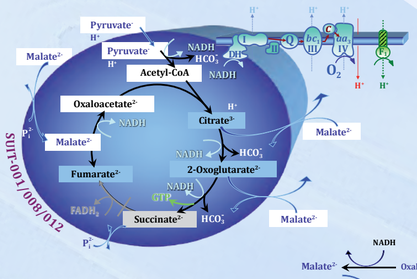
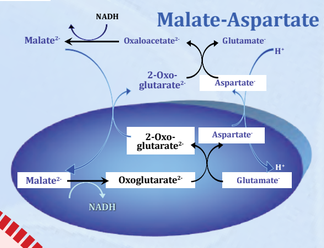


PM

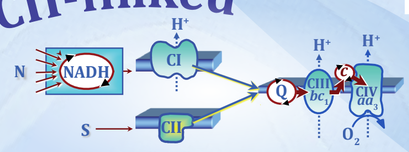


Malate-Aspartate shuttle

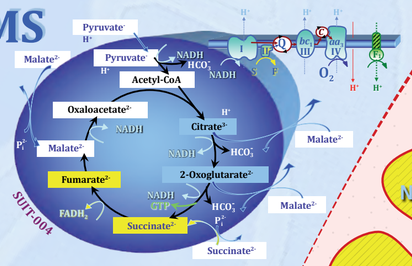


mitochondrial preparations

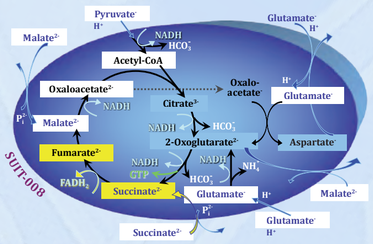
NS-pathway: CI&CII-linked



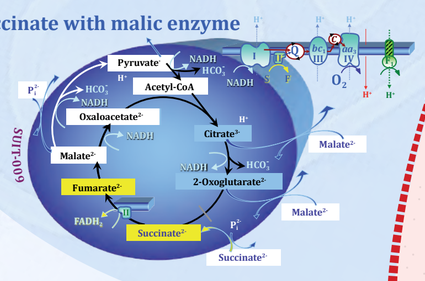
PMS



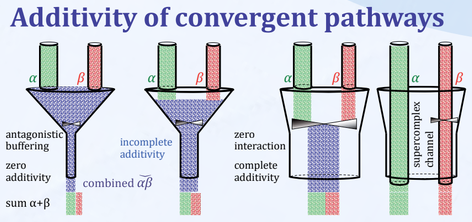
PGMS

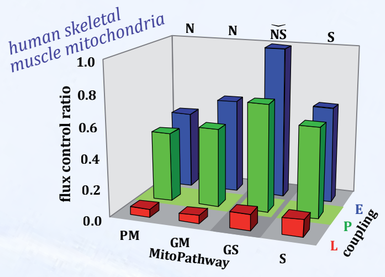


Succinate with malic enzyme

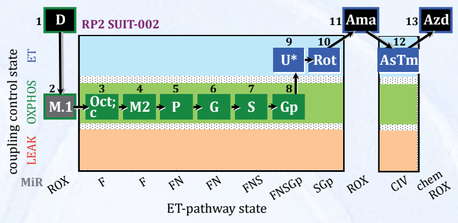


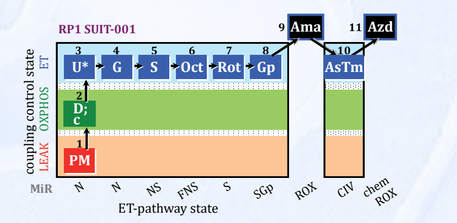
Additivity of convergent pathways



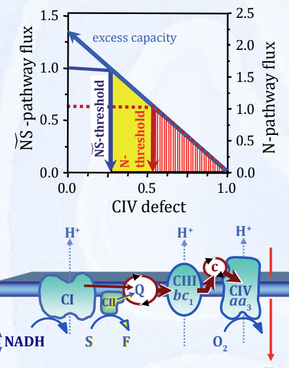


SUIT protocols: OXPHOS fingerprinting





Biochemical threshold: NS- and N-pathway



Pyruvate-H+

Pyruvate- H+ NADH HCO3- Acetyl-CoA CoA-SH

Oxaloacetate2- Citrate synthase Citrate3- Aconitate hydratase Isocitrate

NADH Malate dehydrogenase Malate2- Isocitrate dehydrogenase NADH HCO3- 2-Oxoglutarate2- CoA-SH

TCA cycle mitochondria in the living cell SUIT-003

Fumarate hydratase H2O Fumarate2- Oxoglutarate dehydrogenase HCO3- NADH

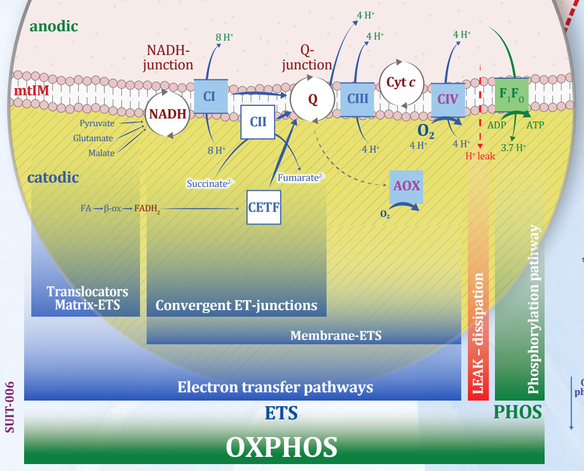
FADH2 Succinate dehydrogenase CII Succinate2- Succinate-CoA ligase GTP ATP CoA-SH Succinyl-CoA

anodic

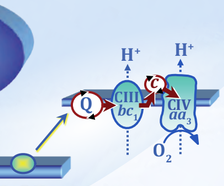
mtIM

catodic

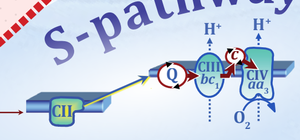
ETS electron transfer system



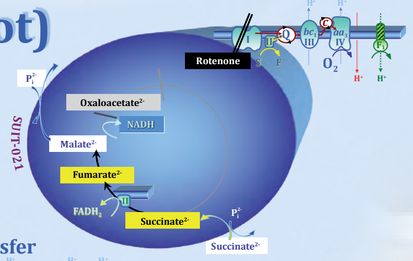
Gp-pathway



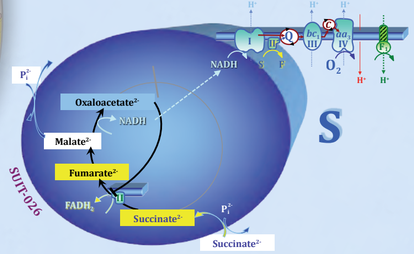
S-pathway: CII-linked



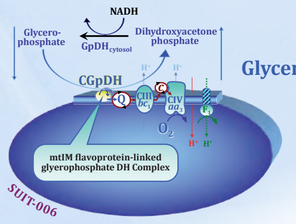
S(Rot)



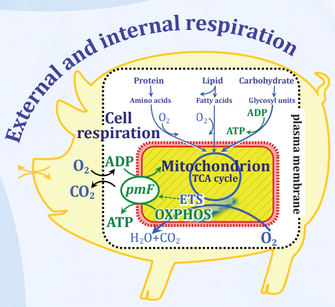
Reversed electron transfer



Glycerophosphate shuttle

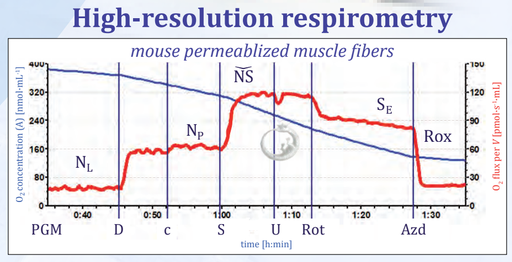


External and internal respiration

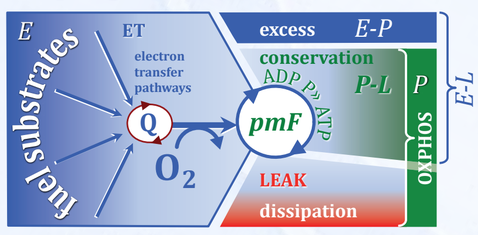


Oroboros O2k

High-resolution respirometry



Coupling states and rates



SUIT protocol and chemicals

Substrates	Event	Concentration in syringe (solvent)	Storage [°C]	Final conc. in 2 mL	Titration [µL]	Syringe [µL]
Pyruvate	P	2 M (H_2O)	fresh	5 mM	5	25
Glutamate	G	2 M (H_2O)	-20	10 mM	10	25
Malate	M	0.4 M (H_2O)	-20	2 mM	10	25
Malate	M	0.05 M (H_2O)	-20	0.1 mM	4	10
Succinate	S	1 M (H_2O)	-20	10 mM	20	50
Octanoylcarnitine	Oct	0.1 M (H_2O or DMSO)	-20	0.5 mM	10	25
Glycerophosphate	Gp	1 M (H_2O)	-20	10 mM	20	50
Ascorbate	As	0.8 M (H_2O)	-20	2 mM	5	25
TMPD	Tm	0.2 M (H_2O)	-20	0.5 mM	5	25
Cyt. c	c	4 mM (H_2O)	-20	10 µM	5	25
ADP+ Mg^{2+}	D	0.5 M (H_2O)	-80	1 - 5 mM	4 - 20	25
ATP+ Mg^{2+}	T	0.5 M (H_2O)	-80	1 - 5 mM	4 - 20	25
Uncoupler						
CCCP*	U	0.1 mM (EtOH)	-20	0.05 µM step	1 µL step	10
CCCP*	U	1.0 mM (EtOH)	-20	0.5 µM step	1 µL step	10
Inhibitors						
Rotenone	Rot	1 mM (EtOH)	-20	0.5 µM	1	**10**
Malonic acid	Mna	2 M (H_2O)	fresh	5 mM	5	**25**
Antimycin A	Ama	5 mM (EtOH)	-20	2.5 µM	1	**10**
Myxothiazol	Myx	1 mM (EtOH)	-20	0.5 µM	1	**10**
Sodium azide	Azd	4 M (H_2O)	-20	≥100 mM	≥50	**100**
KCN	Kcn	0.5 M (H_2O)	fresh	1.0 mM	4	**10**
Oligomycin	Omy	0.01 mM (EtOH)	-20	5 - 10 µM	1-2	**10**
Carboxyatractyloside	Cat	2 mM (H_2O)	-20	1 - 5 mM	1-5	**10**
Other						
Digitonin	Dig	10 mg/mL (DMSO)	-20	10 µg/Mx cells	1 µL/Mx	10
Catalase in MiR06	Ctl	112 000 U/mL	-20	280 U/mL	5	25
H_2O_2 for reoxygenation	Hp	200 mM	fresh		1-3	10

*0.1 mM stock for mt-preparations with high uncoupler sensitivity; 1 mM stock for mt-preparations with low uncoupler sensitivity, living cells in various culture media (e.g. RPMI, DMEM, EGC) and for TIP2k.

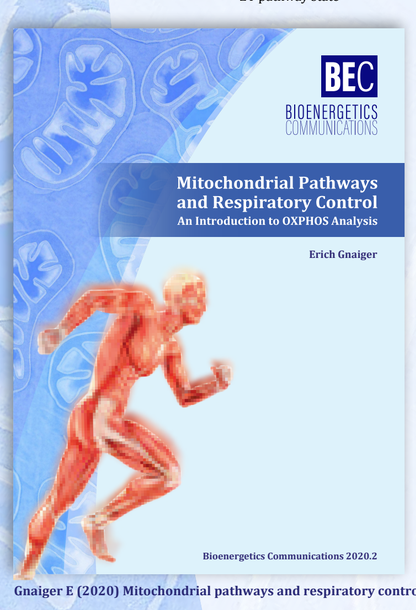
SUIT symbols have evolved for laboratory practice with the requirement to keep abbreviations short. They are not intended for use outside the SUIT framework.

Caution: When using the O2k-sV-Module (small-volume O2k-Chamber: 0.5 mL), titration volumes and stock concentrations have to be adjusted.

Respiratory rates in coupling-control states

Symbol	Definition	mt-Preparations, ET-competent pathway states	Living cells, exogenous or endogenous substrates
rate	J_{kO2} or I_{kO2}		
P **P**	**OXPHOS capacity**, coupled, *pmF* supports J_{P_a}	saturating ADP and P_i or J_{max} from ADP kinetics	can saturating [ADP] and [P_i] be achieved?
R **R**	**ROUTINE respiration**, physiological control of energy turnover in the range from *L* to *P*, variable *pmF*	limiting steady-state ADP levels simulating ROUTINE respiration above *L* and below *P*	physiological control of cellular substrate uptake, intermediary metabolism and energy turnover
E **E**	**ET capacity**, electron transfer capacity, noncoupled, very low *pmF*	optimal uncoupler concentration for maximum respiration	optimal uncoupler concentration for maximum respiration
L **L**	**LEAK respiration**, non-phosphorylating, maximum *pmF*	*L*(n) and *L*(Omy): general; *L*(T): excluding ATPase activity	*L*(Omy)
Rox **Rox**	**residual oxygen consumption**, *pmF* collapsed or driven by reverse F-ATPase	substrate depletion, or inhibition of CI to CIV	inhibition of CI to CIV

P G M S



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